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THE SIGNIFICANCE OF LEUCOCYTES AND STREPTOCOCCI IN THE PRODUCTION OF A HIGH-GRADE MILK.*

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AMONG the many unsettled problems of modern dairying is that of the significance of leucocytes and streptococci in milk. The literature on the question is varied and plentiful and there is a great divergence of the opinions of even the most careful workers and observers. Harris[1] closes his paper on the subject by saying "The time seems ripe for throwing open the whole question for discussion." Especially is this the case since certain municipalities, in view of the evidence in favor of the conclusion that the presence of numerous leucocytes and streptococci, either singly or together, denotes disease in the cow, have felt justified in excluding such milk from sale and in demanding that the producer remedy the matter if he desires to continue supplying the city trade.

That the presence of streptococci or pus in milk is sometimes the visible indication in the milk of disease in the cow injurious to the consumer, no fair-minded person will deny. That the presence of such elements is not always followed by injury to the average consumer is equally apparent. However, from the standpoint of our boards and commissions of health, upon whom rests, very largely, the responsibility of our enormous infantile death rate, there must be formulated regulations which will work the greatest good to the health and well-being of the community and the minimum of hardship to the minority compatible with the attainment of the purpose.

Any inspection of a city's milk supply is difficult, expensive, and annoying because of the ignorance and carelessness of the producer and his propensity for trying to deceive. An inspection involving laboratory work increases all the difficulties. However, an honest endeavor is being made by certain municipalities to carry out laboratory inspection in so far as streptococci and leucocytes are concerned,

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* Received for publication January 9, 1908.

[1] *Jour. Infect. Dis.*, 1907, Supplem. 3, p.

and it is reasonable to suppose that they consider the end to justify the means. They are making an effort, so far as their facilities will permit, to trace the milk to the dairy, and there confirm or deny the laboratory findings.

During 1905 the city of Boston, using the method of Slack for the detection and estimation of streptococci and pus in milk, reported 10.48 per cent of its milk supply thus contaminated. Pus alone was found in 5.01 per cent, pus and streptococci in 1.09 per cent, and streptococci alone in 4.37 per cent, 5,559 samples having been examined. This report expresses the opinion that pus or streptococci render milk unfit for human consumption and indicate disease in the cow. A list of 33 cases is given in which the conditions at the dairy warranted the conclusions reached by the laboratory.

The city of Philadelphia, in 1904, when such inspection was organized, reported pus in 4.2 per cent, pus and streptococci in 0.93 per cent, and streptococci alone in 7.19 per cent, 9,243 samples of milk having been examined; 324 positive findings in the laboratory were confirmed by conditions at the dairies; in 48 cases veterinary inspection failed to show why the laboratory found pus or streptococci in the milk. In 1905, when the work of laboratory inspection was better organized, 9.99 per cent were reported for pus, 2.01 per cent for pus and streptococci, and 11.35 per cent for streptococci alone; 624 of these condemnations were followed to the dairy. The results are given in Table 1.

TABLE I.

A COMPARISON OF THE REPORTS OF THE VETERINARIANS EXAMINING THE HERDS WITH THE FINDINGS OF THE LABORATORY BECAUSE OF WHICH THE INSPECTION WAS MADE.*

VETERINARIAN REPORTED	THE LABORATORY PREVIOUSLY FOUND				
	Pus	Streptococci	Pus and Streptococci	Blood and Streptococci	Blood and Pus
Inflammation (mammitis, garget, etc.).	107	145	48	1	...
Milking too close to parturition.......	22	43	5	...	...
Milking for too long a period.........	5	8	1	...	...
Cow pox...........................	2	1	1	...	...
Out of condition....................	1	11	...	...	...
Wounds of udder....................	7	9	1	...	...
Ropy milk.........................	1	3	...	...	...
Cows were excluded but no reason was given............................	18	38	9	...	...
No disease observed when the examination was made..................	84 (37%)	64 (19%)	13 (16%)	... ...	1 ...
	222	322	78	1	1

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* Report of Bacteriological Laboratory of the Bureau of Health of Philadelphia, 1906.

The returns from these dairy examinations, for both 1904 and 1905, show a certain number stating that "no disease was observed when the examination of the herd was made." Such cases are, for the two years mentioned, about 20 per cent of the whole number investigated. It must not be forgotten, however, that the notification to the farmer was made by post; that in many of the country districts the mail is irregularly delivered or called for, so that delays are always likely to occur on this account. The farmer may also defer calling the veterinarian to make an examination, and it may be a matter not only of days but sometimes of a week or two before the veterinarian reaches the farm to examine the cows in question. Such being the case, it is more than likely that the conditions which prevailed when the milk was examined had either remedied themselves or become so noticeable that the farmer himself was compelled to take them into account. It would seem, therefore, that depending, as the city must, on a tardy, irregular, or incomplete examination of the herd, the percentage of agreement between its laboratory findings and the reported examinations show rather an unusual accord.

The method which has been used for the detection of streptococci and of the number of leucocytes present in the milk is the centrifuge method of Stewart. For an accurate enumeration of the number of cells in milk this method will not serve. For the routine examination of a large number of milk samples, however, when the exact count of the leucocytes is not required, but rather a knowledge sufficient to indicate the fitness of that particular milk for food, we believe the method to be eminently satisfactory. Without doubt the number of leucocytes obtained by this method is lower than, for example, that by the Doane-Buckley method, but up to the present time we have not been able to fix absolutely a numerical leucocyte standard for the indication of disease in the cow; and the diagnosis of such from the sediment of the milk must, until this value is fixed, depend very largely upon the familiarity of the observer with the particular method of enumeration which he is using, and the condition in the animal which, by experience, he finds to parallel his microscopic results.

It is doubtful, too, when the examination of a large milk supply, as for a city, or the examination of the individual cows of a large herd, is under consideration, whether the extra time consumed by the requirements of one of the more accurate numerical methods is not more than overbalanced by the additional number of samples which such a method as Stewart's enables one to investigate. The use of any of the established methods for the study of leucocytes in milk furnishes the person who is examining many samples from many sources with information which is not confined solely to the number of cells per cubic centmeter.

The often-quoted statement of Savage[1] that he "cannot differentiate between a leucocyte and a pus cell" is undoubtedly true so far as the comparatively few isolated cells of a clean milk are concerned. However, it does seem possible, in a large percentage of cases, to determine, by considering the relative number of the various kinds of leucocytes going to make up the cell content of the milk, whether they are polymorphonuclear, large or small mononuclear, eosinophiles, etc.; and by their staining and appearance, whether they are degenerated or in good condition morphologically, loose lying cells or collected into masses with evidences of fibrin; and from such observations it seems possible to determine whether the condition which produces them is likely to be pathological or normal.

It may be that streptococci in milk, from animals showing no clinical signs of mastitis, are not pathogenic to laboratory animals. Kruse,[2] Hölling,[3] Heinemann,[4] and others have put forth the view that the streptococcus found in milk, which they term *Strept. lacticus*, is a constant inhabitant of the cow's udder and is, in reality, the lactic-acid-forming organism of milk. Heinemann, in a later paper,[5] states that this organism, both morphologically, culturally, and in pathogenic property, is closely related to *Strept. pyogenes;* that, in doses of 2 c.c. of 24-hour old broth culture, given intravenously, it is fatal to rabbits, and passed subcutaneously through several animals, its virulence increases so that the above amount is fatal in subcutaneous injections. Petruschky and Kriebel,[6] however, attribute much of the summer infantile diarrhea to these organisms in milk. Escherich[7] has made a lengthy study of streptococcic enteritis in children, finding a greater number of the organisms in the intestines of infants fed on cow's milk than in those of breast-fed children. Lammeris and von Harrevelt[8] report serious illness traced to the *sterilized* milk of a herd in which one cow had had mastitis. She had been clinically well for some days and it was not supposed that her milk was still infected. Jacobsen[1] traced an infection among adults to a herd where four cows showed streptococci in their milk, but the clinical signs were too slight to be observed by the milkers.

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[1] *Jour. Hyg.*, 1906, 6, p. 123.

[2] *Centralbl. f. Bakt. Abt.* 1, 1903, 34. p. 737.

[3] Dissertation, Bonn, 1904.

[4] *Jour. Infect. Dis.*, 1906, 3, p. 173.

[5] *Ibid.*, 1907, 4, p. 87.

[6] *Die Ursachen der Sommersterblichkeit der Säuglinge u. die Möglichkeit ihrer Verhütung*, Leipzig, 1904.

[7] *Jahrb. f. Kinderheilk.*, 1899, 49, p. 137.

[8] *Ztschr. f. Fleisch- u. Milchhyg.*, 1901, 11, p. 114.

It has been observed by one of us in the examination of many samples of milk from various sources made because the children fed on them were either acutely ill or did not thrive, that in a very great majority of the cases a streptococcus was present. Many times the milk was, otherwise, above criticism. Frequently there has been an opportunity to examine each individual cow furnishing the milk, and when the animal giving streptococci was located and excluded the trouble ceased.

We have no positive method for determining before the milk is consumed whether the organisms which morphologically are streptococci are or are not virulent to all animals of greater and of less susceptibility. Therefore, in our present state of knowledge a milk, to be above reproach, must be free from streptococci. The physicians, parents, or nurses sufficiently progressive to have such laboratory examinations made, usually select a source of supply above the average; hence it is an exception to find a cow in such herds having any clinical signs of udder inflammation.

In the past few years four cases have been observed in which the human mammary gland, without any signs whatever of inflammation, yielded, in the milk, pus and streptococci. Three of the infants fed from these breasts were desperately ill when the milk was brought for examination. The other, an infant only two weeks old, was losing weight and had green stools. All the children recovered when taken off the breast.

A large number of leucocytes in milk may not be harmful, but their presence does, sometimes, stand for definite pathological conditions and, therefore, milk with numerous leucocytes may cause digestive disturbances or such obscure disorders as now baffle the pediatrician.

Both pus and streptococci in milk may be detected in the laboratory in a brief space of time and it has seemed to us possible to make the laboratory of very practical service to the dairy in this matter, and also to accumulate some data which cannot be obtained where the lack of harmony is so pronounced as it is between the health boards and the usual dairymen.

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[1] *Ztschr. f. Fleisch- u. Milchhyg.*, 1901, 11, p. 114.

The examination, yearly, of approximately 15,000 samples of milk by the municipal laboratories of Philadelphia and Boston, from all sorts of sources, must furnish results which are entitled to consideration. That such work leaves much to be desired because of the imperfect facilities which now prevail for the correlation of the conditions at the dairy and the results obtained in the laboratory, is equally true. It has been deemed worth while, therefore, to set forth some of the observations made, so far as pus and streptococci are concerned, on a herd which is being maintained for the production, commercially, of a milk of the highest possible quality, around which so many safeguards shall be thrown that it may be used with impunity, unheated, for the most susceptible infant or delicate invalid.

To such an end a dairy consisting of about 70 milking cows sends to the laboratory daily the milk of such a number of cows as will enable the laboratory to examine each individual in the herd weekly. It also sends a sample of mixed milk of the herd. The samples are examined by the Stewart method and the findings communicated promptly to the dairy. If an animal is reported as having streptococci in her milk she is excluded from the herd until the organisms disappear. Streptococci are very seldom found in the cows of this herd, and in almost every instance a reason for their presence has been forthcoming when conditions were analyzed. When pus is reported the cow is examined clinically, and if no evidences of disease are present, her record is looked up with the result, very frequently, that the cause of the pus is made clear.

This system was started about two years ago and so far has been most successful. A study of the records show that there is in the majority of cases a close connection between large numbers of leucocytes in milk and the physical condition of the cow both in relation to and independently of udder inflammation.

We have found, for example, that the close of the period of lactation is very sharply indicated. It is a textbook assertion that pus makes its appearance if a cow is milked too long. That such a condition is recognized widely is indicated by the many reports sent to the Board of Health of Philadelphia stating that, when pus was found, the milking had been too long continued. It was not surprising, therefore, when making consecutive observations on a carefully watched herd, to find that a cow, which throughout her whole period of lactation had given a milk low in leucocytes, should suddenly develop pus, and that such a development indicated the close of her milking period. In almost 75 per cent of the herd, perfectly healthy, clean cows show pus at the close of the milking period and a coincident chemical analysis indicates that the percentage of butter fat is from 0.5 to 1.5 per cent above the normal for that particular individual. Such a condition is interpreted as indicating the termination of lactation, and accordingly the cow is at once dried off with, apparently, advantage to both herself and her calf.

Of some significance also, in the study of increased leucocytes in the milk of clinically perfect cows is the fact that, from the herd which we are watching, 19 cows have been sold during the year 1906, not for specific defects, but because in most cases they were unsatisfactory milkers and were not commercially profitable owing to their comparatively small yield. Of these 19 animals, 15 showed pus in a large proportion of examinations, but had no physical signs of udder trouble. One cow had positive findings seven times between April 1 and April 25, when she was sold; another had eight positive reports within the three months preceding her sale; another showed pus five times in about 12 weeks, and so on.

It is possible that the frequency of excessive numbers of leucocytes in cows of mixed blood which fall below a paying milk yield is dependent to a certain extent upon the ration fed. This must of necessity be as high as is compatible with the health of the herd. It is varied as much as possible and contains always grass pasture or ensilage (the latter being substituted for grass from October to May), and such other foods as alfalfa, clover hay, malt sprouts, sucrene feed, beet pulp, buckwheat middlings, dried brewer's grains, and gluten. There is fed, however, very little gluten. It has seemed to us possible that some cows might respond to the high feeding with increased leucocytosis, rather than with increased milk yield, and this point is one which we hope later on to investigate practically.

If a chemotaxis of this character occurs in cows which are constitutionally small milkers, a study of cows which are, from the standpoint of the dairyman, admirably adapted to the business for which he keeps them, is of interest as affording light on the reverse of the proposition. Accordingly, from their behavior in the barn, their milk yield, and general fitness, the best 10 cows in the herd were selected for study. The laboratory records of these cows showed that in their milk the presence of pus was scarcely ever noted except at the close of lactation or when they were suffering from cow pox; that in not a single one of the 10 have streptococci ever been observed, and some of these animals have been in the herd for three years. Not one of the 10 is a Jersey; only one has any quantity of Guernsey blood and her cross is Durham; and others are Ayrshire, Holstein, and Durham and are, for the most part, thoroughbreds. So striking has been the cleanliness of the milk of the above breeds as compared with that of Jerseys and Guernseys, when kept under just the same conditions, that we propose making a more careful study of this phase of the production of a sanitary milk.

In certain cases we have observed many degenerated leucocytes in milk when there has been a constitutional disturbance entirely foreign to the udder. Such, for instance, was noted in a clean cow which caught a heavy cold accompanied by congestion of the lungs. It lasted for about a month, during which time the milk showed pus. After the cow regained her health, the pus ceased. She never showed the slightest indication, clinically, of udder inflammation.

Probably no greater calamity can befall a dairy herd than the appearance among them of an infectious mammitis, unless it be a tubercular infection. It would seem to be fairly well established that not only can the entire output of milk be contaminated by one animal, but she may be responsible for the illness of the whole herd. We believe that a strict laboratory surveillance of each cow, with a close watch clinically, greatly lessens the likelihood of the spread of such an infection. Pus and streptococci persist in milk in an attack of mastitis after all physical signs have disappeared; and one or the other, or both, appear before clinical signs are manifest. It has been our observation, also, that when a cow has once shown evidences of udder inflammation and continues to exhibit a tendency to a high leucocyte count, she is apt to acquire a streptococcus showing. Such cows are always a source of worry, if of nothing else. For example, a cow, No. 57, shortly after calving, showed pus and a staphylococcus in her milk. This condition cleared up within a week, but excessive leucocytes from time to time persisted with, finally, three consecutive findings of streptococci associated with pus and evidences of udder inflammation. The cow was not very profitable, and to prevent a possible infecting of the herd, she was sold.

This is a condition which has been noted a number of times. Increased leucocytes appear and, without any clinical evidence of disease, persist for weeks. Then streptococci are noticed. With such a history the cow is either quarantined or sent to the butcher, generally the latter, since it has been deemed better to sacrifice what may be a harmless cow rather than to risk infecting the others with a mastitis. In the case of two valuable animals a quarantine has been established and their milk examined daily for months, indeed during almost the entire period of lactation. One such developed an udder inflammation which seemed to be chronic, and the other, tuberculosis.

Among the conclusions reached by Harris in his study of this subject it is stated that, "Particularly, should more attention be given to the veterinary inspection of the cows' udders, with less absolute dependence upon laboratory examination of milk for signs of infectious processes." It is both necessary and desirable that the care of dairy animals be supervised by a veterinarian, and it has been the experience of this dairy that the constant laboratory examinations have stimulated a more careful and close observation of the animals by those having the practical care of them, both the veterinarian and the herdsman. Upon the herdsmen, far more than upon the veterinarian, who could scarcely be expected to examine a large herd daily, must rest the responsibility of the detection of the first signs of disease. Frequently the finding of numerous cells or streptococci indicates to him a course of action which, had he waited for the usual clinical signs, would have been deferred to the detriment of the animal. For example, a cow having been in the herd for more than two years, and possessing a clean laboratory record for that length of time, suddenly gave a milk containing many cells. Examination showed what the milker had failed to see, namely an inflamed spot at the junction of one teat with the udder. This developed into a boil. Five days after the first examination pus and streptococci in abundance were in the milk from the sore teat and streptococci were in the milk from the other three quarters. When the pus was first noted in the laboratory the lesion, clinically, was so inconspicuous that only careful looking found it, and without the pus report, in all probability, it would not have been noticed for several days.

Of considerable interest in this connection is the occurrence of pus in the milk of cows several days before the lesions of cow pox make their appearance. Indeed, the association of cow pox with pus in milk, accompanied occasionally by streptococci, has been an exceedingly interesting phase of the study of this herd. The barns had been unusually free of pox until the autumn of 1905 when one or two cows were found with well-marked lesions. Then it spread rapidly and many cases developed.

A comparison of laboratory findings with those of the herdsmen showed, occasionally, pus reports from cows normally clean several days before pox appeared macroscopically. A typical case of this kind has the following history: A cow examined on November 28 and December 6 gave clean milk; on December 14 pus in quantity was found, and again on December 15 and 16 the same condition prevailed. On December 18 there were signs of pox, which were unmistakable on December 19. This was a severe case and very persistent leaving finally a scaly udder which was difficult to keep clean. The condition did not improve and the cow was sold in August of 1907.

A study of 16 cases of pox, both mild and severe, showed pus in nine of them. Generally this seemed to come from all the quarters, but two cases were found in which one quarter, only, was affected. Five of the 16 cases showed sterptococci with the pus, and in one case, which was very severe, both streptococci in long chains and staphylococci in abundance were observed.

The external evidence is by no means an indicator of the internal condition. Some cows with marked lesions, if milked carefully and with proper precautions, give a milk free from cells; others with mild cases, no matter how carefully they are handled, may persist in giving a milk rich in cells.

A phase of the question of leucocytes in milk which would be of great value is the relationship, if there is any, between their presence there in increased numbers and an invasion of the animal by a tuberculous infection. When the udder itself is the seat of the lesion one would naturally expect evidences of tissue disintegration in the milk, but this would not, of necessity, follow an invasion of other tissues.

We have observed 14 cows which have failed to pass the tuberculin test. The great majority of them, however, though purchased with a tuberculin test certificate, were condemned a few months after buying; hence the laboratory records are short. Of the 14, four never showed an increase of leucocytes; six had scattering leucocyte counts, but not sufficient to cause uneasiness; four had prounonced leucocytosis and had been carefully watched on this account for signs of disease, but none were noted. For three of these cows, two of which were under observation for a longer period than the others, the following brief histories are given:

No. 5. Fresh in October, 1906. Ten successive laboratory examinations, covering three months, showed a clean milk. Then she had pus occasionally, and in the fifth month there was an inflammation of one quarter with pus and a trace of blood in the milk. From this time on until July, when she was condemned for tuberculosis, she showed scattering pus counts, 19 examinations giving eight positive results. At autopsy the tubercular lesions were found in the intestines only.

No. 25. This animal gave pus in her milk every two or three weeks during the whole period of lactation, but there were never any clinical findings to account for its presence. She was dry for two months, then calved normally, but again showed pus frequently. Before the second period of lactation was ended she failed to pass the tuberculin test.

No. 80. Bought November 24, 1906. Blue grade. Fresh. Laboratory examinations began on November 30, when pus in quantity was reported. Between this date and July 6, 1907, her milk was examined twenty times. On December 7 a second pus report from the laboratory caused a careful examination of the cow's udder and it was found that there was an incipient case of pox. This was well developed on December 19, when again pus in quantity was found. A fortnight later the pox was cleaned up and her milk was almost free of leucocytes. Between this time and July 6 15 laboratory examinations were made seven of which showed pus in quantity, but very careful search, clinically, failed to show any abnormality. However, the cow failed to pass the July tuberculin test and was slaughtered. There were no lesions found in the udder.

The foregoing statement of the laboratory findings in so far as streptococci and increased leucocytes are concerned and their correlation, when possible, with the condition of the animal clinically, is of necessity incomplete. The work is in its infancy and the results which we have obtained may be interpreted in various ways. They are offered, however, with the hope that others studying the production of pure milk will give their co-workers the benefit of their observations, since only by broad researches, preferably on herds which are purely milk producing, can some of the questions now unanswered be settled.

Whether laboratory findings are or are not to be rigidly accepted by the herdsman we believe that they tend toward the production of a clean milk.

The milk of the herd which we have reported is examined daily, also, for the number of organisms. It is plated each day when about six hours old, having been sent to the laboratory directly from the farm, and the milk of the same milking is again plated when received from the dealer and is from 26 to 40 hours old. In this way trouble either at the farm or in the hands of the milk dealer in the city can be traced. Each month an average of all the counts made during the preceding four weeks is struck. Table 2 gives such averages for 13 months. These figures represent over 500 examinations.

TABLE 2.

Month	Milk from Dairy 6 hours Old	Milk from the dealer from 27 Hours to 40 Hours Old
1906-October	3,408	3,849
November	3,379	3,060
December	2,372	2,399
1907-January	2,768	2,412
February	4,127	4,881
March	2,426	2,227
April	1,576	1,694
May	2,818	3,997
June	3,554	3,133
July	1,774	2,565
August	2,166	4,400
September	4,218	6,481
October	2,974	1,373

SUMMARY.

A correlation of the finding of streptococci or of many leucocytes in milk by the laboratory, with the physical condition of the cows in a herd maintained for the production of a very clean milk, would seem to show that in many cases there is a connection between such findings and the condition of the cow, both in relation to specific udder and to systemic affections. Such parallelism seems to obtain for the end of the lactation period, for the beginning of an udder inflammation, for an attack of cow pox, and, possibly, for chemotactic conditions due to high feeding of animals constitutionally unable to transform the increased feed into increased milk.

The frequent laboratory examination of the milk of individual cows has materially assisted the herdsman in preserving the good health of the animals and has stimulated more frequent and careful clinical observations.

It is believed, too, that such examinations are a very material factor in maintaining a bacterial count which, for the past 13 months, averages 3,267 organisms per c.c. when the milk is from 26 to 40 hours old.

Our cordial thanks are due to Dr. E. Q. St. John, who has most ably assisted in the microscopic and bacteriological work needed for this paper.